modes of experimentally determined crystallographic structures with computational prediction of the pyrimidine derivative binding to CAs. Structure-thermodynamics correlations will be discussed. Several compounds bound to select CAs with single-digit nanomolar affinities and could be used as leads for inhibitor development towards anticancer target CA isozymes.

Heme Proteins

2868-Pos Board B23

Structural Dynamics of the Signal Transducer Protein HemAT as Revealed by Time-Resolved Step Scan FTIR Spectroscopy

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Aerotaxis is an important biological process for bacteria, as they utilize a signal transduction system to rapidly sense and respond to fluctuating O_2 levels and retain their homeostasis. HemAT isa heme-based O_2 sensor protein that acts as a signal transducer for aerotaxis in *Bacillus subtilis*, controlling the organisms' movement towards increasing O_2 gradients. The molecular mechanisms for intra- and inter-molecular signal transduction processes are largely unknown. In our work, we have employed time-resolved step-scan Fourier transform infrared (TRS^2-FTIR) spectroscopy to investigate the protein structural changes induced by ligand (CO) photodissociation and rebinding that are crucial for understanding the initial events of the intramolecular signal transduction mechanism in HemAT. We have studied the truncated sensor domain and full length HemAT-CO adducts as well as the Y70F, Y133F, L92A, and T95A mutants. Monitoring the kinetics of CO rebinding to the heme-Fe^{2+} (t_d=6μs-6ms) reveals biphasic kinetics for both the full length and truncated sensor domain HemAT. The TRS^2-FTIR experiments have additionally revealed that Y70 predominantly controls the conformational changes that are induced to the protein matrix by CO photodissociation. Moreover, L92 appears to operate as the conformational gate in the migration pathway of photodissociated CO.

2869-Pos Board B24

Dynamic Allosteric Mechanism of Modulation of Oxygen-Affinity in Human Hemoglobin

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The O2-affinities of deoxy- and oxy-hemoglobin (Hb) [KT and KR,] are reduced as much as 102- and >103-folds, respectively, upon binding of potent heterotropic effectors(1). However, the quaternary structures, the stereochemical structures of the heme environment, the heme coordination structures and the axial electronic structures of the heme coordination (1/2 Fe-His = 215 cm-1 or 1/2 Fe-O = 567cm-1)(3), either in crystal(2) or in solution(1,3), show no detectable changes upon binding of these potent heterotropic effectors. Thus, the O2-affinities of Hb [KT and KR] are correlated to neither T/R-quaternary structures nor the ligation states of Hb in the presence and absence of heterotropic effectors. They, further, indicate that these effector-bindings to either deoxy- or oxy-Hb do not alter the O2-affinities of the hemes in the respective ligation states of Hb, though they reduce the O2-affinities of the respective states of Hb. Our molecular dynamics simulation(4) indicates that the amplitudes of high-frequency thermal fluctuations of the protein matrix significantly increase as the O2-affinity of Hb is reduced, independent of the quaternary structures and the ligation states. Bi-molecular ligand association/dissociation processes to/from the hemes in hemoproteins such as Mb and Hb are interfered from physical barriers of the protein matrix (the ''Cage'' effect). The O2-affinity in Hb is regulated by the effector-linked, dynamic modulation of high-frequency thermal fluctuations of the protein matrix in Hb (the dynamic allostery mechanism) rather than static quaternary/tertiary structural changes in Hb.

References:

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2870-Pos Board B25

Roles of Amino Acid Residues in Woolly Mammoth Hemoglobin on the Temperature Effect of Oxygen Binding

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The O_2 affinity of hemoglobin (Hb) is affected by allosteric effectors (e.g. H^+, chloride, and organic phosphate) and temperature. Hb oxygenation is exothermic, i.e., the oxygen-binding affinity increases significantly with decreasing temperature. This makes it difficult for the Hb molecule in blood to deliver oxygen to the tissues under conditions of hypothermia during major surgical operations. We constructed plasmids to express recombinant woolly mammoth Hb (rHb WM) and Asian elephant Hb (rHb AE). Our biochemical-biophysical studies show that the apparent heat of oxygenation (ΔH) of rHb WM is less negative than that of rHb AE and human normal adult Hb (Hb A), suggesting that the O_2 affinity of rHb WM is much less dependent on temperature. In order to investigate the key residues of the Hb molecule responsible for the temperature effect on O_2 affinity, mutants with β/δ101 substitutions (β/δ101Gln→Glu, Lys, and Asp) in rHb WM have been expressed.Compared to rHb WM,these mutants exhibit a higher affinity for oxygen, and a more negative ΔH value under various conditions of pH, temperature, and salt concentration, with and without organic phosphates. Titrations for the O_2 affinity of those mutant rHbs as a function of chloride concentrations indicate a lower heterotropic effect of this anion due to the replacement of β/δ101Gln, suggesting that the β/δ101Gln residue in rHb WM is important for its stronger response to chloride ions, and also responsible for its lower temperature effect of O_2 affinity. NMR measurements for rHb WM and its mutants have been used to correlate their structural and functional properties. These findings could provide new insights into designing hemoglobin-based oxygen carriers (HBOCs) for treating patients undergoing therapeutic hypothermia (e.g. cardiac arrest, traumatic brain injury, stroke, etc.).

2871-Pos Board B26

Time-Resolved Thermodynamics and Transient Kinetics for Oxygen Photorelease from Hemoglobin:Effector Complexes

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Photoacoustic calorimetry and transient absorption have been used to characterize thermodynamic and kinetic parameters for oxygen photorelease from human hemoglobin (Hb) complexed with allosteric effectors in terms of time-resolved volume and enthalpy changes, quantum yields for bimolecular rebinding, and rate constants for oxygen rebinding. Under stripped conditions we observe a significant temperature dependence of enthalpy and volume changes associated with oxygen photorelease. Below 16° C an endothermic enthalpy and volume expansion ($\Delta H = 272 \pm 60$ kcal mol^{-1}, $\Delta V = 19.5 \pm 4.5$ mL mol^{-1}) were determined for O_2 photorelease, while above 16° C the reaction becomes exothermic and a volume contraction is observed ($\Delta H = -252 \pm 79$ kcal mol^{-1}, $\Delta V = -58.3 \pm 16.5$ mL mol^{-1}). Similar temperature dependence of $\Delta V/\Delta H$ was observed in phosphate buffer at ionic strength of up to 0.2 M. Moreover, the reaction volume and enthalpy changes observed in the presence of 500 mM NaCl (I = 0.5 M) are significantly smaller ($\Delta H = -27 \pm 8$ kcal mol^{-1}, $\Delta V = 7.9 \pm 0.7$ mL mol^{-1}) and the temperature dependence is eliminated, suggesting the significant contribution of electrostriction to the observed reaction parameters. L35 also eliminated the temperature dependence without affecting volume and enthalpy changes associated with O_2 photorelease, effectively extending the low temperature trend. L35 is known to bind strongly in the central cavity of Hb in the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ clefts, and may exhibit this effect by modulating intersubunit interactions. IHP acted similarly but decreased related enthalpy changes. In addition, allosteric effectors and phosphate buffers were found to increase the quantum yield for bimolecular rebinding of oxygen, and rate constants for oxygen rebinding to Hb:L35 and Hb:IHP complexes were lower than those for the stripped protein.

2872-Pos Board B27

Oxygen-Dependent Depolymerization of Sickle Cell Hemoglobin Polymers in the Lungs: Kinetic Mechanisms and their Significance for Pathogenesis and its Prevention

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Our kinetic studies have characterized the mechanisms of deoxyhemoglobin S polymer depolymerization when exposed to CO (used as a model for oxygen because rates can be controlled photolytically). Fibers dissolve slowly, losing monomers from their ends at low partial pressure, and very rapidly at higher partial pressures that induce fiber fracture and therefore many new ends. Slow dissolution that is not complete in the time red cells traverse the pulmonary microvasculature will generate residual arterial polymers (RAPs), enhancing pathogenesis by seeding nucleation of new polymers, accelerating repolymerization and increasing its extent.

We now demonstrate, to the best of our knowledge for the first time by direct observation, that anaerobically drawn arterial blood of sickle patients shows birefringence in many red cells and therefore RAPs exist, which we confirm by EM observation of aligned polymers. RAPs exist not only under hypoxemic conditions, when they can be explained by limited solubility due to the presence of deoxyHbS, but also when hypoxemia is absent. RAPs without hypoxemia imply that slow depolymerization kinetics are responsible. One minute of voluntary hyperventilation and (separately) brief nasal oxygen greatly decrease RAPs. RAPs increase during sleep. We attribute these results to accelerated depolymerization at increasing levels of oxygen that cooperatively induce polymer fracture (fracture, using CO, exhibits a 4.7 power dependence on pCO). These results and the interdependent progress of oxygen saturation, partial pressure, fracture rate and remaining polymer that we model bear on pathogenesis and particularly on vaso-occlusive crises, which result from red cell rigidification and from cellular adhesion due to polymer-dependent cellular damage. Under these mechanisms, the lungs may play an important role in initiating pathology; and remediation of dysfunction by breathing assists is potentially prophylactic.

2873-Pos Board B28

Sickle Cell Therapy and the Kinetics of Polymerization in the Presence of Ligands

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Sickle cell disease results from a mutation of normal human hemoglobin that renders it capable of polymerization once oxygen is delivered. The process of deoxygenation involves quaternary as well as tertiary changes in Hb, and both changes appear to be involved in permitting HbS to assemble into the stiff polymers that distort cells and lead to the occlusion of the microvasculature. Kinetics are central to the disease, because if polymerization is slow enough to occur in the veinous return, the polymerization can be reversed upon reoxygention with minimal if any consequences. We have recently completed a detailed study of the polymerization kinetics of HbS in the presence of ligands. We have found that the kinetics are consistent with equilibrium measurements that show singly ligated HbS molecules will polymerize with only about 0.35 the probability of a deoxy HbS molecule. Given knowledge of these highly concentration sensitive rates, and a distribution of intracellular concentrations, we can calculate the likelihood of sickling at various points in the microcirculation. Because of the finite rate of oxygen delivery, we find sickling is most likely at the exit of the capillaries, which is where obstruction has been observed with intravital microscopy. When this is combined with the results for mixtures of fetal hemoglobin (HbF) or HbA, it becomes possible to determine therapeutic targets for the reduction of rates of sickling.

2874-Pos Board B29

Biophysical Studies Evaluating the Potential Physiological Relevance of the Hemoglobin Associated Nitrite Anhydrase Reaction as a Pathway to Generate S-Nitrosothiols from Low Levels of NO and Nitrite

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Hemoglobin (Hb) has been implicated in nitrite-mediated mechanisms that generate bioactive forms of nitric oxide by the reaction of deoxy Hb with nitrite to produce NO (Nitrite Reductase) is viewed as part of the mechanism since the generated NO is readily scavenged, raising questions as to how free NO could escape from the red blood cell. A proposed nitrite anhydrase reaction (NA) between met-Hb and both nitrite and NO to yield N_2O_3, a potent S-nitrosating agent capable of generating longer lived S-nitrosothiols, could address this limitation. Concerns regarding the physiological relevance of the NA reaction stem from the low affinity binding of nitrite to met-Hb and the competition of reductive nitrosylation which generates NOHb. We have identified a relatively stable spectroscopically distinct species generated from met-Hb and the combination of NO and nitrite that can S-nitrosate glutathione. We have tentatively assigned this species to the purported NA intermediate in which N_2O_3 is bound to the heme. The intermediate can be efficiently generated under conditions of low NO and low nitrite. We find that when NO binds to met- Hb, the affinity for the subsequent binding/reaction of nitrite dramatically increases. using sol-gel matrices to trap R and T forms of Hb, we find that for the T state the reductive nitrosylation pathway is favored, whereas for the R state the NA pathway is favored implying a control mechanism for the production of S-nitrosothiols via the NA pathway. Similar studies using HbE, a mutant Hb having an enhanced redox potential, support a mechanism whereby the R/T dependent redox potential is the primary factor that controls the partitioning between the RN and NA reactions of Hb.

2875-Pos Board B30

Modulation of Nitric Oxide Reactivity by Heme Posttranslational Modification in the Cyanobacterial Hemoglobin, GlbN

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Synechococcus sp. PCC 7002 is a model cyanobacterium capable of thriving under conditions that promote the buildup of reactive oxygen and nitrogen species (ROS/RNS). This organism harbors a hemoglobin (GlbN) that is thought to aid in the detoxification of RNS including NO.

GlbN achieves hexacoordinate heme (FeIII/FeII) binding using His70 (proximal) and His46 (distal). In vitro, this coordination scheme protects against H2O2-induced damage, facilitates electron transfer (ET), and lowers redox potential. Under reducing conditions, His117 attacks the 2-vinyl group to form a covalent crosslink. The irreversible posttranslational modification (PTM) of GlbN yields GlbN-A. Ligands such as CO, O2, and NO inhibit the facile PTM. This and other observations suggest that both GlbN and GlbN-A are active in the cell. How does the His-heme PTM influence GlbN reactivity towards NO?

NMR and optical spectroscopies were used to study the differences in NO binding, NO oxidation, ET, and NO reduction. We observed that GlbN and GlbN-A can form unusually stable diamagnetic FeIII-NO complexes. Both FeII-O2 proteins appear capable of NO dioxygenation, where ET is typically rate-determining. Each GlbN exhibits facile ET, with measured self-exchange rates slightly slower than cytochrome b5. A difference in NO reactivity is observed under strongly reducing conditions: surprisingly, unmodified GlbN is capable of reducing NO to nitrosyl hydride (HNO). Additionally, FeII-NO binding in GlbN results in immediate heme loss.

The results provide some insight into the ability of GlbN to protect the cyanobacterium from RNS/ROS. The data suggest that GlbNs can serve as NO dioxygenases, but may not require a dedicated reductase because of their propensity for facile ET. Additionally, an unusual NO reductase-like activity may also exist for GlbN, and the His-heme PTM appears to eliminate this chemistry.

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2876-Pos Board B31

Redox-Controlled Proton Gating in Bovine Cytochrome *C* Oxidase

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Cytochrome *c* oxidase is the terminal enzyme in the electron transfer chain of essentially all organisms that utilize oxygen to generate energy. It reduces oxygen to water and harnesses the energy to pump protons across the mitochondrial membrane in eukaryotes and the plasma membrane in prokaryotes. The mechanism by which the oxygen reduction reaction is coupled to proton pumping remains unresolved, owing to the difficulty of visualizing proton movement within the massive membrane-associated protein matrix. Here, with a novel hydrogen/deuterium exchange resonance Raman spectroscopy method (1), we have identified two critical elements of the proton pump: a proton loading site near the propionate A group of heme *a*, which is capable of transiently storing protons uploaded from the negative-side of the membrane prior to their release into the positive-side of the membrane and a conformational gate that controls proton translocation in response to the change in the redox state of heme *a*. These findings form the basis for a new molecular model describing the mechanism, by which unidirectional proton translocation is coupled to electron transfer from heme *a* to heme a_3 associated with oxygen chemistry occurring in the heme a_3 site during enzymatic turnover.

2877-Pos Board B32

Reduction of Iron Center Enhances the Mechanical Stability of Cytochrome B562

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Because redox energy can be converted into conformational energy, heme redox proteins offer a unique opportunity to examine the coupling between redox reactions and protein mechanics. Here, we use Atomic Force Microscopy-based single-molecule force spectroscopy (SMFS) to directly examine the effect of heme and its oxidation state on the mechanical properties of cytochrome b562 (cyt b562). We found that cyt b562 is mechanically stronger in its reduced state as compared to its oxidized state. In addition, we discovered the shortening of